LUNULATION AND GENETIC ANALYSIS IN *ARICIA* BUTTERFLIES

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Abstract

Lunulation in *Aricia* species (Lep.: Lycaenidae) was studied in Durham and Hampshire during 2003. Data from this fieldwork, as well as existing data from sites in the Peak District and Yorkshire, are discussed. Comparisons are drawn with European data. Previous conclusions drawn from Peak District data are confirmed. The status of a Yorkshire population and the nomenclatural aspects of all the studied populations is discussed with reference to the overall *Aricia* picture. A history for the genus is reiterated.

Introduction

The first paper giving details of genetic analysis on butterflies from several *Aricia* colonies in Britain and Scandinavia appeared recently (Aagaard *et al.*, 2002). Its findings were discussed with Andrew Pullin, one of the joint authors, and he mentioned that 'there is considerable agreement between lunulation and geographic variation in allele frequency as shown by allozyme analysis.' The analysis confirmed that the Scottish race *Aricia artaxeres artaxerxes* was very similar to European *A artaxerxes allous*, so that the Scottish race has been formed from *allous* and is not peculiar to Scotland. Also the presence of *artaxerxes* in the north of England was confirmed – again there is no special endemic race *salmacis*, but the analysis was unable to clarify whether hybrids were or were not present. A subsequent more sophisticated analysis, as yet unpublished, was able to confirm hybrids in the north of England (Wynne, pers. comm., 2002). For the above areas genetic analysis and conclusions via male upper forewing lunulation (mufl) are in agreement. The grey areas remaining in the east of England are the status of colonies near Pickering, north Yorkshire, and in the Peak District, where in the Aagaard *et al.* paper there were contradictory results from allozyme analysis (*artaxerxes*) and mitochondrial data (*agestis*).

A poster and information sheet at Butterfly Conservation's fourth International Symposium in Lancaster, during 2002, gave examples of phased emergence in *Aricia* colonies. During any flight period the mufl drifts downwards. Therefore, genetic analysis from samples taken early and late in the same flight period from any site might show up differences as indicated by differences in lunulation. This had already been noted in a Peak District colony, which has *agestis* lunulation (Smyllie, 1992a). As far as is known, specimens for genetic analysis have been taken from any site on one day, a perfectly normal approach. The possibility of carrying out small-scale genetic analysis experiments from males early and late in a flight period at suitable localities was discussed, but found not to be feasible. It was therefore decided to visit a colony in Durham, and another in the south of England, at intervals during 2003 to collect more lunulation data.

Durham

The Durham visits to Thrislington National Nature Reserve (TH) and Bishop Middleham quarry (BM) are summarised below and compared with 1990 figures via Sam Ellis. Visits, with numbers of males in brackets, were made on 4 June (TH-11), 23 June (TH-3, BM-7), 9 July (BM-5, TH-0) and 15 July (BM-0). The situation was disappointing in that numbers had dropped to a total of only five males counted on 9 July and none were seen on 15 July. Normally the flight period continues right through July. Since the butterfly is univoltine there was no second chance. There was little difference between the two sites, and not enough data to check phased emergence. Results show that 2003 was less well lunulated than 1990, and in both 1990 and 2003 all 5th or 6th lunules were only traces. This is important since the overall lunulation is poorer than the actual numbers of 5&6 mufl specimens might indicate. Table 1 summarises the position in 1990 and 2003.

Table 1. Male lunulation at sites in Durham.

YEAR	PERIOD	MC	MP	MT	MC/MT
1990	17-28.6	8	14	22	0.364
2003	4.6-9.7	4	22	26	0.154

MC=well lunulated (5 or 6mufl): MP=poorly lunulated (0-4mufl): MT=total.

Although the results are disappointing it can be seen that there is a distinct difference between the two sets of figures. This is not unusual regarding variations encountered in the north of England. The shortened flight period in 2003 may well have altered the genetic bank by removing at least part of the less well lunulated portion, thereby causing the overall lunulation to increase next year.

Winchester

Magdalen Hill Down (MHD) is a Butterfly Conservation nature reserve to the east of Winchester with a south-facing slope approximately 1 mile long. The first brood was again disappointingly short, but some data was obtained. The second brood behaved more normally, and Table 2 gives details of all visits with male lunulation, time spent on site and weather.

Examination of the data shows that Nos. 1 and 6, the first days for both broods, have distinctly higher MC/MT figures than the rest. In all but one case the P males had 4ufl, the remaining male on 10.9 had 3ufl. Some of the 4ufl had small or trace lunules. Counting was (in order of preference) basking, placing in a bottle and waiting for the butterfly to settle down and open its wings – this could take up to 20 mins. or longer - catching in a net and trapping with open wings. In order to avoid duplication the lunules were split into large, medium, small and trace. Also comments like fresh, worn, chip out of wing, some white discal spots etc. were made. In practice only one potential duplicate was noted. The numbers of butterflies on site were not large and were spread out, so it was hard work finding them and then counting where possible.

Table 2. Male lunulation at MHD

No.	Date	MC	MP	MT	Time	Weather
1	19.5.03	16	2	18	10.30-12.20	Dull then rain then sun
2	6.6.03	-	-	-	18.00-19.00	Heavy rain
3	8.6.03	2	2	4	13.25-15.10	Some sun: cold wind
4	17.6.03	-	-	-	17.45-19.20	Some sun
5	19.7.03	-	-	-	14.00-15.30	Sunny
6	5.8.03	7	1	8	2-3pm:17.45-19.20	Sun: very hot
7	20.8.03	5	4	9	17.00-19.30	Grey: sun after 19.00
8	30.8.03	8	6	14	14.20-16.50	Mainly grey: some sun
9	10.9.03	5	3	8	14.25-17.30	Sun early and late

Conversely both Marbled Whites and Chalk-hill Blues were abundant over flight periods of four weeks or longer. Details are summarised below.

A	MHD '03 Nos.1 and 6:	MC 23	MP 3	MT 26
B	MHD '03 Nos. 3,7,8,9:	MC 20	MP15	MT 35 14.259 25.741
C	Watlington Hill 20.5.89	MC 8	MP 0	MT 8
D	Watlington Hill 29.5.89	MC 7	MP 2	MT 9
Totals	A+C+D	MC 38	MP 5	MT 43 27.217 34.643

The statistical formula to check whether variation is due to chance or some other factor is np +/- k√(npq) where n is the total number = MT, p = MC/MT, q = MP/MT = p-1 and k is a constant. Five percent "significance limits" are employed as a standard. When k=1.96 and numbers are outside the limits there is a less than 5% likelihood that the difference is due merely to chance, so a more than 95% likelihood that some other factor is involved.

The first records for both broods at MHD, nos 1 and 6, have been added together to give A. All other counts later in the broods are somewhat lower in MC and are added together to give B. In order to increase the early totals, C and D from Watlington Hill, Oxon. have been added to A. Without these additions the figures are not large enough to provide adequate separation. The two right hand columns above give the calculated 5% significance limits for the B total of 35. The corresponding figures for A+C+D have been reduced from 43 to 35 for comparison. There is a gap between the two close limits of 27.217 – 25.741 = 1.476. Not large, but one which will increase if further data were to be accumulated. This indicates that the earlier and later portions of one brood at an *agestis* site in south England vary by an amount which is greater than that which can be attributed to chance. The same situation will apply for males at any *agestis* site.

A. *agestis* and the Peak District

The initial impetus to examine lunulation came from a comment (Jarvis, 1969) that all *agestis* colonies were well lunulated whereas north of England colonies were variable and lunulation decreased further through Scotland. The standard for well lunulated males was set at 5 or 6 upper forewing lunules (mufl) whereas better lunulated females required 6fufl. Table 3 shows 5&6 mufl (MC) but also 6 mufl for field counts at Coombs Dale (CD) in 1992 and other years excluding 1992 in order to provide a comparison with MHD. Neither set of CD figures gives a 5% significance separation, but the main point is that both CD and MHD show reductions in lunulation when the first and second halves of the broods are compared. Early on in the flight period the well lunulated portion can be up to 100%. For example, the weekly checks at CD in 1992 for just one week shorter than in Table 3, i.e from 18.5 to 21.6 (four visits) showed that 18 out of 18 males had 5 or 6ufl. On this showing, admittedly from a relatively small number, it might be claimed that CD was a *cramera* colony. It might even be claimed that the figure of p=0.958 up to 28.6 from 23/24 males was still high enough to indicate *cramera*. This is not a serious claim because of the later reduction, but it does indicate the type of difficulty which phased emergence can cause. Study of the figures shows a fair amount of variation. Also the CD 6mufl figures could be on the whole lower than MHD. This might indicate a greater *artaxerxes* content than MHD without the general lunulation pattern being altered.

Table 3. Coombs Dale and Magdalen Hill Down lunulation.

LOCALITY	MC/MT	P	6mufl/MT	P
Coombs Dale 31.5-28.6.92	23/24	0.958	14/24	0.583
Coombs Dale 5.7-2.8.92	20/27	0.741	7/27	0.259
Total	43/51	0.843	21/51	0.412
Coombs Dale up to 30.6.89-95	19/23	0.826	9/23	0.391
Coombs Dale after 2.7.89-95&02	23/36	0.639	8/36	0.222
Total	42/59	0.712	17/59	0.288
Magdalen Hill Down 19.5 & 5.8.03	23/26	0.885	15/26	0.577
Magdalen Hill Down 8.6 &20.8-10.9.03	20/35	0.571	15/35	0.428
Total	**43/61**	**0.705**	**30/61**	**0.492**

It is very important to indicate, if possible, the main reasons for variation at any site presuming no migrating butterflies reach the colony. The Meteorology Office 30 year statistics give the average hours of sunshine, also maximum and minimum temperatures on a monthly basis for stations around the UK and Europe. There is a considerable difference in the hours of bright sunshine between say Bournemouth, Buxton and Braemar which for May, June and July total 661, 487 and 474 hours respectively On average Bournemouth is between 3° and 3.5° warmer than the other two each month. These differences are large enough to indicate that the effect on lunulation is likely to be extremely small, otherwise there would be much larger and more random variations due to this factor in some of the *agestis* sites. The main other candidates are predation, unfavourable weather at critical times in the larval or other state, and phased emergence. Taking one year at a time, it is quite possible that either separately or in combination all could play their part. As is the case in Durham, a partial first brood this year has been a disappointment, but indicates the probable major source of subsequent variation. There will be much less lunulation change later on at MHD compared with Durham, so any effect on *agestis* will be much smaller.

Restricting comments to *agestis*, the data banks contain approximately 850 males and 550 females. When males are split into single or small groups of counties, all are within the 5% significance limits of the average (Smyllie, 2001). The Peak District is included in this list. So, in spite of the annual variations noted, the bulk figures have smoothed these out. Nearly all of the specimens providing the data have come from museum collections, and there is probably a tendency to collect early in a brood in the expectation of greater numbers of fresh specimens. The 2003 figures for males at MHD are a little outside the statistical limits, but this merely covers variation during one single season with multiple visits. Even then the MC/MT figure of just over 70% is considerably higher than the next group of relatively well lunulated colonies at c55% max. These will be considered with Pickering.

Each year is likely to be different as far as numbers of butterflies, completeness of brood, and random predation goes. At the female level of consistency it is not possible to separate different colonies via female lunulation from the south coast up to and including the Pickering district (Smyllie, 1992b). It follows that it is not possible to get meaningful variation in female lunulation through the flight period. The female lunulation is not only higher than the male, it is also less prone to produce the odd specimen with trace lunules in southern colonies. So possible operator judgement as a variable factor in deciding whether a trace is or is not present is low in males and rare in females.

Therefore, presuming that there is a link between DNA and lunulation, a difference between male and female DNA at ag*estis* sites is to be expected, provided samples are not taken very early on in the flight period. This aspect might provide an explanation for the difference noted for the Peak colonies in the Aagaard paper. This does not appear to be the case however for other *agestis* material analysed. At this point an unfortunate aspect in the paper has to be mentioned. Fig. 3 plots lunulation characteristics and indicates via symbols whether the haplotype involved is *agestis* or *artaxerxes*. Fordon Bank (Yorkshire Wolds) lunulation is MC 90%(11), FC100% (10). Figures in brackets denote sample size. These figures are exactly as expected from accumulated lunulation data. The Peak District colonies are Coombs Dale MC 64% (28), FC 67% (9), and for Cressbrook Dale MC 25% (39), FC 40% (8). It is the female figures for both colonies and the male from Cressbrook Dale which give the most cause for concern – all 60 Peak District females in the author's data bank have 6 ufl. There must therefore be a question mark against the validity of these samples. Possibilities are unauthorised introductions – these have occurred from time to time in the last 20 years in the general area of Derbyshire, Notts. and Yorkshire – or sample mixing.

The wider *Aricia* picture

Tables 4 and 5 have been produced to show the male lunulation position at selected sites. Table 4 figures start with the standard 5&6 mufl since previous papers have largely concentrated on these. Figures for 6 mufl are also included for the first time and are much more searching. It can be seen that they have decreased from 100% (p=1.0) in the Canaries (and almost certainly in parts of Spain) down to less than 60% (p<0.6) at Casa de Campo near Madrid, Table 4/2, both the above being considered as *cramera*. This site is only some 75 km east of the Sierra de Gredos mountain range, Table 5/13), and will have been affected by its relative proximity. It has to be remembered that we are talking about interpenetration shortly after the last ice-age, not last year. Looking at combined figures, the *agestis* examples from England and the Peak District are distinctly lower (p=0.5-0.3) while all other examples are well under p=0.3 and reduce to 0, numbers 7-17 all being p<0.1 or =0.

Table 4. Lunulation from selected sites.

NO. LOCALITY	5&6 mufl	p	6 mufl	p	0 mufl	p
1 Tenerife, Canaries	11/11	1.0	11/11	1.0	0/11	0
2 Nr Madrid, Spain	70/73	0.959	41/73	0.562	0/73	0
3 S England	208/252	0.825	103/252	0.409	0/252	0
4 Peak District	125/157	0.796	54/157	0.344	1/157	0.006
5 near Pickering	18/44	0.409	10/44	0.227	0/44	0
6 Kaiserstuhl, SW Germany	22/40	0.55	9/40	0.225	1/40	0.025
7 inland Durham NZ33	12/50	0.240	2/50	0.04	1/50	0.020
8 Skane ex Sandhammaren	10/20	0.5	1/20	0.05	1/20	0.05
9 Chiasso, TI, Switzerland	5/18	0.278	0/18	0	2/18	0.111
10 Sandhammaren, S Sweden	15/62	0.242	2/62	0.032	9/62	0.145
11 N Lancashire	41/210	0.195	10/210	0.048	58/210	0.276
12 coastal Durham	16/177	0.090	3/177	0.017	56/177	0.316
13 SW Scotland	7/35	0.200	3/35	0.086	7/35	0.200
14 SE Scotland	3/47	0.064	0/47	0	15/47	0.319
15 Scotland N of Forth to Inverness	10/286	0.035	0/286	0	164/286	0.573
16 Hirsthals, N Denmark	1/20	0.05	0/20	0	11/20	0.55
17 Scotland N of Inverness	0/13	0	0/13	0	10/13	0.769

Whereas Table 4 deals with the picture at the higher lunulation end of the spectrum, Table 5 has some overlap and continues down to the poorest lunulation. It consists mainly of Scandinavian data * (Høegh-Guldberg, 1966) together with nos. 3&4, the author's south England and Durham data, which act as a link with Table 4 for lunulation. The right-hand column gives the % with no lunules on any upper wing, var. *unicolor*. This column has not been previously included. Nos. 12 and 13 were so poorly lunulated on the upper forewings that hind wings were also noted. The broad picture is of a complete range of 6 mufl from 100% to 0% between the Canaries and the north of Scotland, with, at the other end of the scale 0 mufl from 0% to 100% and with %s of var. *unicolor* from 0% up to c60%. Note that it is relatively easy to see small numbers of lunules at the bottom end of the scale, even if they are faint. At the other end it is not possible to judge a small diminution in the lunule size of a well lunulated male which will still have 6 ufl.

Table 5. Scandinavian and mountain lunulation

NO. LOCALITY	4-6 mufl	p	0 mufl	p	V unicolor	p
1* Skane ex Sandhammaren	44/55	0.800	0/55	0	0/55	0
2* Sandhammaren	103/175	0.589	1/175	0.006	1/752	0.006
3 south England	242/252	0.960	0/252	0	-	-
4 Inland Durham NZ33	38/50	0.760	1/50	0.020	-	-
5* Oland	9/24	0.375	1/24	0.042	0/24	0
6* Gotland	7/24	0.292	0/24	0	0/24	0
7* Denmark *agestis*	93/122	0.762	0/122	0	0/122	0
8* Jomfruland, S Norway	6/42	0.143	7/42	0.167	0/42	0
9* Hirsthals, N Denmark	5/239	0.021	58/239	0.243	24/239	0.100
10* Lyngenfjord, N Norway	0/9	0	7/9	0.778	2/9	0.222
11* Uppland, C Sweden	0/19	0	6/19	0.316	2/19	0.105
12* Angermanland, N Sweden	0/8	0	6/8	0.75	5/8	0.625
13 Sierra de Gredos, C Spain	0/13	0	13/13	1.0	1/13	0.077
14 Haldenstein, Swiss Alps	2/19	0.105	17/19	0.895	5/19	0.263

Near Pickering and similar areas

The colonies near Pickering are typically at Ellerburn and Pexton Banks at the southern fringe of the north Yorkshire moors, some 18 km north west of the Yorkshire Wolds. The 5&6 mufl is distinctly lower (Tables 4 and5, approximately 40%) than the *agestis* colonies with a minimum of 70%. The female lunulation however is unchanged at c100% with 6 fufl. Occasionally the apical lunule is not much more than a trace, but nevertheless female lunulation provides no segregation. The following genetic comments are relevant (Wynne, pers. comm., 2002): 'In this connection mtDNA has only one quarter the effective population size of nuclear genes (e.g. allozymes)) and this contributed to a clear line between haplotypes some 150 km north of the univoltine-bivoltine boundary. Information re hybrids came via a nuclear gene'. From the lunulation point of view, the fact that males are a better sex for comparison has an echo in genetic analysis.

Re. the gap of 15% between the lower *agestis* 5&6 mufl limit of 70% and the next colonies at c55%, the inference is that there is a certain stability about *agestis* which enables its lunulation to be unchanged from the south coast up to the Yorkshire Wolds: one would expect a trend of increased *artaxerxes* moving north from the south coast but, if present, this has not altered the lunulation pattern appreciably.

Table 6 shows the make-up of figures for the Pickering area. It mostly contains small numbers (3 to 8) which can be at completely opposite ends of the lunulation spectrum. The demarcation line is between 4 and 5 mufl, and Table 7 shows the much higher occurrence of 4 mufl compared with south England and the Peak District.

Table 6. Pickering data.

Museum	Page	Collection	Locality	Date	MC	MP	MT
Keighley	M1	CR Haxby	Pickering	1958	8	12	20
Scarboro'	M2	E Richards	Newton Dale	1.8.55	0	3	3
Peterboro'	M3	Cabinet N	Pickering		1	1	2
Doncaster	M10	A Norris	Gundale		1	0	1
Leicester	M18	Smith	Pickering		4	2	6
"	M19	CP Pickett	"		0	8	8
"	M20,21	A Lisney	"		4	0	4
Field	-	B Smyllie	Ellerburn Bk	9.7.01	4	0	4
Totals					22	26	48

Without last row p = 18/44 = 0.409. With last row p = 22/48 = 0.458

The above data indicate that there are distinct differences between Pickering, Kaiserstuhl etc. and the *agestis* colonies as far as 5&6 or 6 mufl is concerned. When data from any colony like Pickering is examined, variability as met in Table 6 is also a feature. The other end of the spectrum is the position at 0 mufl where at Table 4/5 none are recorded out of 48. So, if the decision was based on good lunulation, Pickering and similar sites would be categorised as intermediates. On the other hand, classifying via 0 mufl would be more lenient, thus expanding the *agestis* area, and including areas in different countries where *agestis* is already nominated.

Take Baden-Württemberg (BW) in south-west Germany where *agestis* and *artaxerxes* are both recorded and mapped, with the additional comment that hybrids are also found (Ebert & Rennwald, 1991). The shadow from the Alps extends a long way northwards down the Rhine valley. In the NW corner of BW the colonies near Mannheim, c230 km north of the Swiss border are little different to Kaiserstuhl in Table 7, and it is not until Mainzersand, a little west of Mainz at 270 km, that *agestis* figures similar to those in the UK are recorded. However, only one 0mufl specimen (totals in brackets) was recorded for Kaiserstuhl (41) and none for Weinheim/ Mannheim (37). So it may be expedient to define *agestis* colonies as those which have

Table 7. Pickering and Kaiserstuhl 4 mufl

LOCALITY	MC/MT	p	4 mufl/MT	p
South England	208/252	0.825	34/252	0.135
Peak District	125/157	0.796	29/157	0.185
Pickering	22/48	0.409	18/48	0.375
Kaiserstuhl	22/40	0.55	15/40	0.375

a suitably low 0 mufl content, rather than those which are within the standard statistical 5 & 6 mufl limits using p=0.825 from larger numbers. Just as in the UK *agestis* sites, lunulation will be at its maximum early on in the flight period and to the lepidopterist in the field this will show up as well lunulated. The type of variation shown by Table 6 is typical of Pickering and similar colonies.

Discussion

It is best to start by examining the present position concerning lunulation and genetic analysis. Until relatively recently species have been identified by morphological characteristics; the advent of genetic analysis has provided an opportunity to apply a cross-check from a different angle. It is very important to determine if variable morphological characteristics do or do not match up to analysis. From the lunulation point of view, this means, firstly, that the data built up to record variation has to provide meaningful rather than random information and secondly that conclusions arising from scrutiny of the data have also to be correct. When aspects of the development of a genus over tens of thousands of years are attempted there is plenty of room for a degree of deviation from what will eventually be found out by expanding knowledge and techniques. In all this the data remain constant and will provide a pole-star reference. Aagaard *et al* (2002) did not cover Pickering, and as mentioned above the Peak District samples have a question mark against them, particularly since the genetic results for the Yorkshire Wolds further north come into line with lunulation.

It is reasonable to make two predictions – first that enough of the general picture has been pieced together so far to indicate that morphological and genetic information will eventually be seen to coincide and second that morphological characteristics provide a more sensitive indicator than genetic analysis in its present state of development. Information via morphology will provide comparative data without being able to give firm percentages on the amount of interpenetration.

The lunulation checks were originally built up to compare the Peak District race with other *agestis* colonies, later with other UK areas. This is important because it led to 5&6 mufl being selected as a yardstick for good lunulation, and with over 80% of males in *agestis* colonies having this lunulation there is an understandable tendency to regard *agestis* as one end of the spectrum. It is as far as the UK is concerned, but later data from Spain and the Canaries gives a wider backdrop to the situation. This is where the more stringent 6 mufl data is important. The well lunulated *agestis* is now by no means so well lunulated on the absolute scale, and even *cramera* does not have a uniform lunulation. Everything is part of a progression from 100% to 0% or 0% to 100% for the two opposite ends of the lunulation spectrum. It does therefore seem necessary to highlight an aspect of *Aricia* previously mentioned (Smyllie, 2001). The two races which drifted apart to reach no lunules and a full set of lunules on the upper wings are represented today by *cramera* and *allous/artaxerxes/morronensis*. This means that genetic analysis should be able to compare well lunulated *cramera* with poorly lunulated *allous* and follow the variation through the intermediate stages. So far *cramera* has not featured in the equation via genetic analysis as far as the author is aware. It is therefore desirable to obtain analytical data for *cramera*.

Nomenclature

Aricia nomenclature would be a fertile ground for a politician – there are different aspects to be taken into account so that good cases can be made out for most alternative names. It is worth considering these in order to examine the variables involved. First of all there are the two extremes *cramera* and *allous* (or equivalents). The initial problem is to decide how much penetration from the other end can be allowed: if for example the figures were to be 10% at each end, this would leave 80% to account for. Figures of 5% would leave 90% while greater than 10% at each end are likely to be too high. Assuming that 10% is about right, this still leaves 80% to be allocated. The simplest solution is just to have one name, *agestis*. This however covers a wide range, some of which has fairly obvious morphological differences. So the next step would be to split the whole range into 4 with *agestis* covering the well lunulated portion and some other name covering the remainder. Provided the general mechanism is understood a number of names can be put forward – *salmacis*, *montensis*, intermediates or hybrids to name a few. It is felt that splitting the range into 4 would be the best option, and also that defining *agestis* as having less than 10% of 0 mufl would be better than taking a stricter line on limits imposed by 5&6 or 6 mufl. In practice the 0 mufl could be set at no more than 1 in a minimum of 20. This will give 5% while 2 would give 10% and be regarded as too high. There are 2 quite good reasons for this suggestion.

1 In practice it would split the remaining 80% more evenly.

2 Several countries, eg Denmark and Germany, already attach the name *agestis* to areas which, like Kaiserstuhl, would not fit the 5% significance limits based

on the well lunulated UK colonies. It would classify the colonies at Pickering and at Perthichwareu in N Wales (Smyllie, 1992a) as *agestis*. Switzerland also has *agestis* and this still presents a problem because of the proximity of these better lunulated colonies to the Alps. Samples (Smyllie, 2001) labelled from Orvin (2 with 0 mufl out of 9) and Chiasso (2 with 0 mufl out of 16) would both fail the *agestis* test via 0 mufl. As a general rule it would not be possible to move from either *cramera* to hybrids or *allous/artaxerxes* to *agestis* without the intermediate stage having occurred. The word 'hybrid' is used reluctantly. It has the advantage of being short, but can give the impression that hybrids only occur over one part of the four, rather than throughout.

Alternative ranges are as in Table 8: there may be occasions in the 4-stage situation where a colony does not fit into designated limits in both columns: in any such case, the general situation and colonies nearby have to be considered. Also it may be possible for variation over a period of time to change the status of the 'hybrid' colonies. Incidentally the possibility of very varied lunulation at one site has been attributed to *agestis* and *allous* co-existing (Høegh-Guldberg, 1966). This has been investigated and found not to occur (Smyllie, 1998). What does occur is phased emergence.

Table 8. Nomenclature limits.

'sub-species'	5&6 mufl%	0 mufl%
cramera	90 min.	10 max.
agestis	90-30	10 max.
'hybrids'	30-10	10-30
allous	10 max.	30 min.

Summary and conclusions

1 Genetic analysis and mufl data show considerable agreement, so it is reasonable to assume that lunulation data can make a positive contribution to residual grey areas in the UK and also to the overall situation in Europe west of Switzerland including Scandinavia. The same basic principles will apply anywhere for this genus.

2 The data bank is very stable, particularly for those areas with larger numbers.

3 In looking at the longer term history of the genus *Aricia*, previous conclusions (Smyllie, 2001) are reiterated

4 Interpenetration and phased emergence are fundamental aspects of a coherent explanation for *Aricia*.

5 Lunulation is backed by other morphological aspects such as white discal scales (Smyllie, 1992b), variation in ocelli pupillation (Smyllie, 1997), and egg reticulation (Smyllie, 2001)

5 The interpenetration is between *cramera* and *allous* or its equivalents. The sequence with brief comments is: *cramera* - some over-lunulated females are found at least as far north as Durham (Jarvis, 1969): *agestis* – stable lunulation over large areas; 0 mufl less than 10%: 'hybrids' – poor lunulation and/or 0 mufl 10%+: *allous* – very poorly lunulated

6 Eventually genetic analysis and lunulation etc. will line up though more development in analysis will be necessary.

7 The situation in several other butterfly species will be similar to *Aricia* though not obvious morphologically. Small White, *Pieris rapae* (Smyllie, 1997) and Common Blue, *Polyommatus icarus* are examples.

8 The factors involved should be useful in enlarging practical knowledge about butterflies generally, rather than being looked on as an abstract theory.

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